REMARKS/ARGUMENTS

Claims 20-27 are pending in the above-referenced patent application and are currently under examination. In order to expedite prosecution, Applicants have amended claim 20 to pursue more focused subject matter. Support for the amendments to claim 20 can be found throughout the specification as filed, including, *e.g.*, in Example 2. Example 2 describes, in great detail, culturing lineage committed mammalian cells with a test compound in a culture medium; subsequently removing the test compound and culture medium; and then culturing the cells in a first and second cell differentiation medium, *e.g.*, ODM (osteogenic differentiation medium) and ADM (adipogenic differentiation medium). As such, no new matter has been introduced with the amendments to claim 20. Reconsideration is respectfully requested.

There is only a single rejection remaining in the present case. In the Office Action, claims 20-27 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly non-enabled. For the reasons set forth below, Applicants respectfully traverse this rejection.

Rejection Under 35 U.S.C. § 112, First Paragraph:

Claim 20-27 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement. Specifically, the Office Action alleges that the claims contain subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention without undue experimentation. Applicants respectfully ***disagree***, and to the extent this rejection is applicable to the newly amended claims, Applicants respectfully traverse this rejection.

In an earnest effort to expedite prosecution and without acquiescing on the merits of the rejection, Applicants have amended claim 20 to pursue more focused subject matter. As amended, claim 20 is directed to a method for identifying compounds that induce dedifferentiation of **mesenchymal** lineage committed mammalian cells into multipotent stem cells. In this method, a **mesenchymal** lineage committed mammalian cell (such as an adipocyte, a myoblast, an osteoblast, a chrondrocyte, *etc.*) is cultured in the presence of a test compound suspected of inducing dedifferentiation of lineage committed cells. The test compound and

culture medium are then removed. Thereafter, the cells are cultured in a first cell differentiation culture medium that induces differentiation of the multipotent stem cell into a first cell type and in a second cell differentiation culture medium that induces differentiation of the multipotent stem cell into a second cell type. It is then determined whether the cells have undergone differentiation into the first or second cell type, wherein induction of differentiation into both the first cell type and the second cell type identifies the test compound as a compound that induces dedifferentiation of lineage committed mammalian cells. Thus, claim 20, as amended, recites:

> 20. A method of identifying compounds that induce dedifferentiation of mesenchymal lineage committed mammalian cells into multipotent stem cells, said method comprising
>
> (a) culturing, in a culture medium, the mesenchymal lineage committed mammalian cells with a test compound suspected of inducing dedifferentiation of the mesenchymal lineage committed mammalian cells for a time sufficient to induce dedifferentiation to multipotent stem cells;
>
> (b) removing the test compound and the culture medium;
>
> (c) culturing cells of step (b) in a first cell differentiation culture medium, wherein the first cell differentiation culture medium induces differentiation of the multipotent stem cells of step (b) into a first cell type;
>
> (d) culturing cells of step (b) in a second cell differentiation culture medium, wherein the second cell differentiation culture medium induces differentiation of the multipotent stem cells of step (b) into a second cell type;
>
> (e) determining whether the cells of step (b) have undergone differentiation into the first or second cell type, wherein induction of differentiation of the cells of step (b) into both the first cell type and the second cell type identifies the test compound as a compound that induces dedifferentiation of lineage committed mammalian cells.

As those of skill in the art appreciate, the developmental potency of a cell exists on a continuum ranging from the lineage committed or differentiated cell, which has limited developmental potency, to a completely developmentally potent cell or totipotent cell, which can give rise to any embryonic or extraembryonic cell type. In between these two extremes, however, are pluripotent cells, multipotent cells and unipotent cells (*see*, *e.g.*, Jaenisch and Young, *Cell* 132, 567-582 (2008), a copy of which is attached hereto as Exhibit A).

The term "pluripotent" refers to the ability of a cell to form all lineages of the body or soma (*i.e.*, the embryo proper). For example, an embryonic stem cell is a type of pluripotent stem cell that is able to form cells from each of the three germs layers, *i.e.*, the ectoderm, the mesoderm and the endoderm, but not extra-embryonic cells. The term "multipotent" refers to the ability of a stem cell to form **multiple cell types of one lineage**. For example, mesenchymal stem cells are capable of forming cells of the mesenchymal cell lineage, including, for example, osteocytes, myocytes, adipocytes, chondrocytes, hematopoietic cells, fibroblasts, stromal or tendon cells. The term "unipotent" refers to cells that can form a single cell type. For example, spermatogonial stem cells are only capable of forming sperm cells.

Those of skill in the art, both now and as of the priority date of the instant application, would appreciate that the multipotency of the presently claimed multipotent stem cells can be determined by testing whether such cells can be induced to differentiate into more than one type of **mesenchymal** lineage committed cell type. Those of skill in the art, both now and as of the priority date of the instant application, would further appreciate that, once committed, a mesenchymal lineage committed cell is not multipotent and, in fact, is only capable of developing into a particular cell type or related cell type (*see, e.g.*, paragraph [0040] of U.S. Publication Application No. 2007/0254884). For example, a lineage committed cell of the osteoblast cell lineage includes osteoprogenitor cells, pre-osteoblasts, and osteoblasts. Osteoblasts then mature into terminally differentiated cells called osteocytes.

Accordingly, a test compound that induces a mesenchymal lineage committed cell to become a multipotent stem cell, which can **then** differentiate into more than one mesenchymal lineage committed cell type, identifies the test compound as a compound that induces dedifferentiation of mesenchymal lineage committed mammalian cells.

For instance, in the present case, the specification clearly demonstrates that culturing a murine myoblast cell line, *i.e.*, C2C12 cells, which are mesenchymal lineage committed mammalian cells, in the presence of Reversine, *i.e.*, a 2,6-disubstituted purine, induces dedifferentiation of the C2C12 cells. This is clear because after the Reversine and culture medium are removed, the resulting dedifferentiated C2C12 cells are **then** able to

differentiate into **both** osteogenic cells when cultured in osteogenic differentiation medium (ODM), as assessed by alkaline phosphatase staining (ALP), **and** adipogenic cells when cultured in adipogenic differentiation medium (ADM), as assessed by Oil Red O staining.

Moreover, others in the field have demonstrated that the effects of Reversine are **not** limited to the dedifferentiation of murine myoblasts. In fact, Applicants provide post-filing evidence that both murine 3T3E1 osteoblasts and human primary skeletal myoblasts treated with Reversine can each differentiate into both osteogenic and adipogenic cells.

For instance, in one nonlimiting example, Saraiya *et al.* (*Tissue Engineering, Part A*, a copy of which is attached hereto as Exhibit B), demonstrate that treatment of mesenchymal lineage committed human annulus fibrosus cells with Reversine induces the cells to become multipotent stem cells that can then differentiate into osteogenic cells, adipogenic cells and chondrogenic cells.

Further, in another nonlimiting example, Fania *et al.* (*Electrophoresis* 30, 2193-2206 (2009), a copy of which is attached hereto as Exhibit C), demonstrate that treatment of murine fibroblasts with Reversine induces the cells to become multipotent stem cells that can then differentiate into skeletal muscle, smooth muscle and bone cells.

The Office Action alleges that the specification fails to provide adequate guidance and evidence for how to identify multipotent stem cells of different cell types derived from numerous different cell types derived from numerous different mammals, and how to identify numerous different cell types differentiated from said multipotent stem cells derived from various mammals. Applicants respectfully ***disagree***. Those of skill in the art would appreciate, both now and as of the priority date of the instant application, that identifying the numerous mesenchymal lineage committed cell types across various mammalian species can be accomplished by using routine histological and morphological criteria. For example, a mammalian adipocyte can be identified by Oil Red O staining, a mammalian osteoblast can be identified by alkaline phosphatase staining, a mammalian muscle cell can be identified by the presence of myosin, a mammalian smooth muscle cell can be identified by the presence of smooth muscle myosin, *etc.* In addition, contrary to the allegations in the Office Action, it is

pointed out that the phenotypic characteristics of the various mesenchymal lineage committed cell types are evolutionarily conserved across mammalia.

Moreover, as noted above, Applicants respectfully point out that the present claims are directed to the identification of a compound that induces dedifferentiation of mesenchymal lineage committed mammalian cells into multipotent stem cells. Again, Applicants respectfully submit that the skilled artisan can identify mesenchymal lineage committed mammalian cells, *e.g.*, osteocytes, myocytes, adipocytes, chondrocytes, hematopoietic cells, fibroblasts, stromal, or tendon cells, **without** undue experimentation. Furthermore, those skilled in the art would easily identify the presently claimed multipotent stem cell as a cell that can differentiate into more than one type of mesenchymal lineage committed mammalian cell.

Further, cell culture media capable of differentiating multipotent stem cells into mesenchymal lineage committed cells were known in the art at the time of filing the present application (*see*, *e.g.*, U.S. Patent Nos: 5,942,225 and 5,736,396, as well as the present specification as filed, including Examples 2-4).

The Office Action further alleges that the presently claimed test compound should be "a differentiation compound" instead of "a dedifferentiation compound." However, in view of the foregoing discussion, one having ordinary skill in the art could only reasonably conclude that the claims are properly directed to a method of identifying a dedifferentiation compound, *i.e.*, a compound that is capable of inducing dedifferentiation of mesenchymal lineage committed mammalian cells into multipotent stem cells.

In view of the foregoing, Applicants respectfully submit that the specification, as filed, provides more than adequate support and enablement for the instant claims in the manner provided by 35 U.S.C. § 112, first paragraph. Accordingly, Applicants respectfully request the Examiner to reconsider and withdraw the rejection under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

Further, the Commissioner is hereby authorized to charge any additional fees or credit any overpayment in connection with this paper to Deposit Account No. 20-1430.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

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